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Review

Reduction of *Campylobacter* spp. by Commercial Antimicrobials Applied during the Processing of Broiler Chickens: A Review from the United States Perspective

OMAR A. OYARZABAL*

Department of Poultry Science, Auburn University, Auburn, Alabama 36849-5416, USA

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ABSTRACT

A reduction in *Campylobacter* spp. has been associated with use of commercial antimicrobial technologies during the processing of broiler chickens. This review is focused on commercial interventions that have received approval by both the U.S. Food and Drug Administration and the U.S. Department of Agriculture for use on raw poultry in the United States. Most of these interventions are currently applied prechill. The limited number of publications on the topic suggests that the application of antimicrobials in commercial settings results in *Campylobacter* reduction of 1 to 2 log CFU/ml of carcass rinse. However, postchill counts of 0.5 to 1 log CFU/ml of carcass rinse (approximately 4,000 CFU per carcass) are still common. Thus, antimicrobial interventions are not a complete solution for the control of *Campylobacter* on raw poultry. New postchill interventions are needed, as are (i) improvements in the methodology for detection and enumeration of *Campylobacter,* (ii) additional surveys on the contamination of processed poultry, and (iii) an understanding of possible resistance to antimicrobials by *Campylobacter* spp. Research addressing these topics will lead to better control of *Campylobacter* in commercial poultry carcasses.

There is a high prevalence of *Campylobacter* spp. in retail chicken carcasses *(36, 52).* The lower parts of the intestinal tract, especially the ceca, of commercial marketage broiler chickens are frequently colonized with high numbers of *Campylobacter* spp., primarily *Campylobacter jejuni (57).* Lower numbers are found in the crop, liver, and respiratory tract *(28, 32, 57).* During processing, the primary contamination of chicken carcasses is believed to originate from fecal material of the chickens themselves *(62).* The spilling of as little as 5 mg of cecal contents containing *Campylobacter* is sufficient to increase the concentration by 0.6 log CFU/ml of carcass rinse in prechill carcasses *(29).* Cross-contamination also may occur during different processing steps *(69),* and chickens from *Campylobacter*-free flocks may acquire contamination from *Campylobacter*-positive flocks *(54).*

Different chemical interventions have been developed to reduce foodborne pathogens in poultry *(15, 35, 37),* but few are used commercially by the poultry industry. Most of the studies performed with commercial compounds have focused on the reduction of generic *Escherichia coli* (nonpathogenic) and *Salmonella* and less on the reduction of other pathogens, such as *Listeria monocytogenes, Campylobacter* spp., and *Clostridium.* Many of the compounds used to control pathogens in raw poultry products are considered processing aids and are approved by the U.S. Food and Drug Administration (FDA) under the Code of Federal Regulations (CFR) title 21, part 173, as secondary direct food additives permitted in food for human consumption. These substances are used in food as antimicrobial agents as defined in 21 CFR section 170.3(o)(2), and their applications have a temporary technical effect in the treated food. Under the proposed conditions of use, these substances are ordinarily removed from the final food, and any residuals that may be carried over to the final product are not expected to have any technical effect *(18).* Other compounds, such as chlorine and trisodium phosphate (TSP), have received approval as GRAS (generally recognized as safe) substances under 21 CFR part 182.

Even though the U.S. Department of Agriculture Food Safety and Inspection Service (USDA FSIS) has no definition of processing aid in its labeling regulations, the FSIS makes judgments on a case-by-case basis using FDA's definition of a processing aid to determine whether a substance is a processing aid or an ingredient of a food. If a substance is considered a processing aid, it does not need to be declared in the ingredients statement, and there is no provision for its use in any standard of identity applicable to the final food *(15).* For the approval of these products, the FSIS usually requires trials where the population of generic *E. coli* and the prevalence of *Salmonella* serovars are determined on carcasses before and after the treatment. For online reprocessing approvals, the comparison is between traditional reprocessing techniques done off-line and the new on-line reprocessing technology. Pretreatment counts usu-

* Author for correspondence. Tel: 334-844-2608; Fax: 334-844-2641; E-mail: oyarzoa@auburn.edu.

TABLE 1. *Commercial antimicrobials commonly used by the poultry industry to reduce* Campylobacter *spp. during processing*

Antimicrobial	FDA approval (21 CFR section)	Use	Suggested mode of action
Acidified sodium chlorite[a]	173.325	Spray of dip solution: 500–1,200 ppm sodium chlorite and any GRAS acid to achieve a pH of 2.3–2.9; prechiller or chiller solution: 50–150 ppm sodium chlorite and any GRAS acid to achieve a pH of 2.8–3.2	Broad-spectrum germicides; oxychlorous compounds act by breaking bonds on cell membrane surfaces
Cetylpyridinium chloride[a]	173.375	Not to exceed 0.3 g/lb of poultry, propylene gylcol concentration 1.5 times that of cetyl pyridinium chloride	Hydrophilic portion reacts with the cell membrane, resulting in the leakage of the cellular components, disruption of cell metabolism, and ultimate cell death
Chlorine (sodium hypochlorite)[b,c]		20–40 ppm in chill water; also used in processing water at 10–30 ppm	Oxidation of cell components resulting in cell death
Chlorine dioxide[a]	173.3	Not to exceed 3 ppm in poultry process water contacting whole fresh carcasses	Oxidation of the cellular membrane and cellular constituents; at high concentrations, it breaks the cell wall
Ozone[b]	184.1563	Antimicrobial agent as stated in 21 CFR 170.3(o)(2),[d] used in gaseous or aqueous phases	Direct molecular reaction and indirect reactions involving free radicals, oxidation of cell membrane
Peroxyacetic acid[a]	173.37	Maximum concentration of 220 ppm peroxyacetic acid, 110 ppm hydrogen peroxide, and 13 ppm 1-hydroxyethylidene-1,1-diphosphonic acid	Strong oxidation of cell membrane and other cell components, resulting in cell death
Trisodium phosphate[b]	182.1778	8–12% solution in conjunction with a water spray containing 20 ppm chlorine; solution can be applied by spraying or dipping chilled or prechilled carcasses for up to 15 s	Disruption of cell membrane causing leakage of intracellular fluid; details of the antimicrobial mechanism have not been completely elucidated

[a] Secondary direct food additives.
[b] Generally recognized as safe (GRAS).
[c] FSIS considers the application of up to 30 ppm of chlorine on poultry to be sanctioned under the food additive provisions of the Federal Food, Drug and Cosmetic Act *(2).*
[d] Antimicrobial agents: substances used to preserve food by preventing growth of microorganisms and subsequent spoilage.

ally define the initial bacterial load on the carcasses to be sampled.

New technologies have resulted in significant improvements in the safety of meat and poultry in recent years. The FSIS defines new technology as ''new, or new applications of equipment, substances, methods, processes, or procedures affecting the slaughter of livestock and poultry or processing of meat, poultry, or egg products'' *(20).* The FSIS has encouraged improvement and innovation in food safety technologies and provides a summary on its Web site that describes some of the new technologies that have been or are under review *(20).* These technologies can be used with no objection by FSIS-inspected establishments *(20).* Unfortunately, the efficacy of these technologies for controlling *Campylobacter* spp. is not completely known, nor has their efficacy been documented in scientific journals. This review contains a summary of the impact of key processing steps on the carriage of *Campylobacter* by broiler chicken carcasses and a discussion of the current scientific data on *Campylobacter* reduction by products approved to control pathogens in raw poultry under 21 CFR parts 173 and 182 (Table 1).

KEY PROCESSING STEPS

After the bleeding process, most of the carcasses positive for *Campylobacter* spp. have a considerable number of *Campylobacter* cells on their skins *(24).* Before scalding, feathered carcasses may contain *Campylobacter* at 5.4 log CFU/g *(24)* and 7.5 log CFU/g *(51)* in their feathers, and the breast skin may contain between 3.8 log CFU/g *(24)* and 6.9 log CFU/g *(51).* The lower part of the intestine, primarily the ceca, is heavily colonized by *Campylobacter* in the range of 6 to 7 log CFU/g *(24, 55, 57).* Crops also have a large prevalence of contamination *(32),* with up to 5 log CFU/g *(24).* However, carcasses whose organs are not contaminated may still harbor *Campylobacter* at 4 log CFU per carcass *(24).*

Scalding. Berrang and Dickens *(26)* found *Campylobacter* counts of 4.7 log CFU/ml of rinse in prescalded carcasses and 1.80 log CFU/ml of rinse in carcasses after scalding. The use of lower temperatures in the scalding water (51 to 52°C, soft scalding) does not result in any significant decrease in the number of *Campylobacter* on carcass skins, but a scalding temperature of 58°C or above, which is typically used in processing establishments in the

United States, yields a significant reduction of *Campylobacter* on carcasses *(43)*. However, a significant increase in the number of *Campylobacter* per milliliter of carcass rinse occurs after defeathering *(26, 43)*. The pressure applied to the carcass during this process may force fecal material out through the cloaca, from where it can contaminate the exterior of the carcass *(57)*. Swab sampling of turkey skin revealed the presence of *Campylobacter* on the skin after defeathering *(1)*.

In studies by Yang et al. *(79)*, scalding temperatures of 50 and 60°C produced reductions of 1.5 and 6.2 log CFU/ml in water, respectively, and <1 and >2 log CFU/cm^2 on chicken skins, respectively. The killing effects of temperature were less apparent for bacteria attached to the skin. There was a more resistant *Campylobacter* population in the scalding water at 55°C than there was at 50°C. *C. jejuni* concentration displayed a tailed curve, with a rapid decline in the first 1 to 3 min and then no changes during the rest of the treatment time. The age of the scalding water (in hours) had no effect on the heat sensitivity of *Campylobacter* during scalding, but the increase in the age of the chilled water significantly reduced the bactericidal activity of chlorine *(43)*. Raising the scalding temperature and the chlorine concentration of the chilled water was effective in reducing *Campylobacter* cross-contamination through water but had little effect on the bacteria attached to the skin *(26)*. Postscald treatments of hot water applied gently enough not to produce any alteration in carcass quality were not effective in lowering *Campylobacter* counts immediately or 30 min after scalding *(27)*.

Evisceration. Practices during the grow out stage, such as feed withdrawal times, play an important role in reducing fecal contamination during evisceration. A short feed withdrawal time, i.e., less than 6 h, results in intestines that are full of fecal material, whereas a long feed withdrawal time (longer than 14 h) results in thinner intestinal lining, which makes the intestines more likely to break during extraction. Fresh eviscerated carcasses may carry up to 10^6 *Campylobacter* cells per carcass *(74)*. The Nationwide Broiler Chicken Microbiological Baseline Data Collection Program reported an incidence of *Campylobacter* spp. of 88.2% in postchill carcasses, with an average of 1.3 log CFU/ml of rinse *(3)*. Although the average number of *Campylobacter* cells per milliliter of carcass rinse may have decreased, the incidence of *Campylobacter* spp. in postchill and retail carcasses is still more than 80% *(36, 52, 58)*. *Campylobacter* concentrations of 2.83 log CFU/ml *(58)* and 3.7 log CFU/ml *(45)* after evisceration and before carcass wash are not unusual in commercial processing facilities, with an incidence of 90% or more carcasses positive for *Campylobacter* *(58)*.

Carcass washers. The effectiveness of carcass washers for reducing *Campylobacter* spp. depends greatly on water volume, water pressure, and the concentration of chlorine in the water *(22)*. These variables may be difficult to control consistently in commercial processing environments and may account for the erratic results obtained with carcass washers. Recent studies have revealed that although *Campylobacter* contamination may drop due to carcass washers, the reduction is neither consistent nor significant *(22)*. In one study, the efficacy of inside-outside carcass washers and homemade cabinet washers was evaluated in groups of one to three per plant. These systems used 9.73 liters of water per carcass and between 25 and 35 ppm total chlorine. Although *Campylobacter* concentrations were reduced by 0.5 log CFU/ml by the washers, the average concentration after the washer was still 4.3 CFU/ml of carcass rinse *(22)*. In another study, researchers indirectly showed that a carcass washer produced modest reductions of *Campylobacter* contamination (between 0.3 and 0.7 log CFU/ml) and left the carcasses with a high incidence (above 90%) of *Campylobacter* *(58)*.

Immersion chiller. Since the 1950s, chlorination has been used in poultry processing water and chiller water in the United States. The main goal was to improve the quality and shelf life of poultry products to make them competitive in the marketplace *(15, 39)*. The chilling operation decreases the carcass temperature to less than 40°F (4.4°C) within a few hours, depending on the weight of the carcass. Rapid chilling limits the growth of spoilage and pathogenic bacteria, thereby increasing the product's shelf life. Depending on the extent of their attachment, bacteria present on the surface of carcasses entering the chiller are frequently removed during the chilling process, as seen by consistent reductions in bacterial numbers in carcass rinse samples measured pre- and postchill *(15)*.

In one study, the chiller accounted for a significant decrease in *Campylobacter* numbers *(58)*. However, a large number of postchill carcasses remained positive for *Campylobacter* spp. after the enrichment of the samples, suggesting that (i) *Campylobacter* numbers are usually high on carcasses and the normal chilling step is not effective enough in reducing them *(58)*, or (ii) the chiller may be a focal point for bacterial cross-contamination *(53, 77)*.

In broiler carcasses, *Campylobacter* has been associated with chicken skin, and removal of the skin early in the processing pathway reduces the numbers of *Campylobacter* on the outside of broiler carcasses, but has no effect on the numbers of *Campylobacter* on the internal surfaces of the carcass *(25)*. In a study where skin was inoculated with *C. jejuni* and *Salmonella* Typhimurium at two concentrations, low (10^3 CFU/cm^2) and high (10^6 to 10^7 CFU/cm^2), the survival curves of *C. jejuni* and *Salmonella* Typhimurium were basically parallel regardless of the inoculation concentration. The authors concluded that the initial concentration of bacterial cells did not affect the reduction in numbers of bacteria on the skin during chilling *(79)*.

CARCASS REPROCESSING AND ANTIMICROBIAL APPLICATIONS

Fecal material is an important source of carcass contamination. Ingesta also has been suggested as a contamination source, although the correlation between ingesta and pathogen load on carcasses is weak *(31)*. Washing with chlorinated water, trimming, and vacuuming have all been used extensively to remove contamination from carcasses

before immersion cooling. These reprocessing steps, called off-line reprocessing, have been implemented to comply with the FSIS stipulation that carcasses contaminated with visible fecal material cannot enter the chill tank.

With the development of antimicrobial compounds, carcasses contaminated with fecal material can now be treated with these agents on the processing line as long as the application of these agents ensures that the contaminants do not enter the chill tank. Therefore, the zero tolerance for fecal material must be met by these treatments to obtain FSIS approval for on-line processing. During these applications, called on-line reprocessing, agents are applied to every carcass prechill regardless of the presence of visible fecal contamination *(15).* In the United States, antimicrobial agents are used as prechill applications for on-line reprocessing, are added to chill tanks to reduce microbial cross-contamination, or are applied postchill. In all cases, the main goal of the antimicrobial agent is to reduce bacterial pathogens. The compounds are applied with spraying cabinets or dipping tanks.

ASC. When acidified sodium chlorite (ASC) is combined with organic matter, several oxychlorous antimicrobial compounds are produced. These products are broad-spectrum germicides that act by oxidizing sulfide and disulfide bonds on cell membrane surfaces *(46).* The FDA has granted approval of ASC as a secondary direct food additive permitted in food for human consumption *(9).* The USDA cleared ASC for antimicrobial treatment of poultry products on 7 January 1999 *(10).*

ASC is used as a carcass spray or dip solution before the immersion of the carcass in the prechill or chill tank. The solution must contain sodium chlorite concentrations between 500 and 1,200 ppm and any GRAS acid at concentrations sufficient to achieve a solution with a pH of 2.5 to 2.9. Citric acid is commonly used. When ASC is used in a prechill or chill tank, the additive is used at concentrations that result in sodium chlorite concentrations between 50 and 150 ppm *(9).*

Kemp et al. *(45)* found that the combination of bird washers with ASC sprays removes fecal contamination and allows for continuous on-line processing of commercial broiler carcasses. Carcasses that underwent continuous on-line processing had a reduction in *Campylobacter* spp. of 1.75 log CFU/ml compared with carcasses that underwent off-line reprocessing. In another study, a reduction of 99.2% in *Campylobacter* numbers was achieved by the combined effect of bird washers and ASC sprayed prechill, but there was no difference in *Campylobacter* numbers between post-ASC and postchill carcasses *(47).* The authors determined that contamination of the carcasses occurred in the chill tank, as had been previously suggested *(53).*

A recent application of ASC is as a postchill dip application. The application of ASC postchill significantly and consistently reduced the concentrations and incidence of *Campylobacter* spp. to less than 0.2 log CFU/ml of carcass rinse. The effect of ASC may be indirectly increased by the stress imposed on *Campylobacter* cells by the chilling process *(58).*

CPC. On 2 April 2004, the FDA amended 21 CFR section 173.375 to allow for the use of cetylpyridinium chloride (CPC) in food as an antimicrobial agent to treat the surface of raw poultry carcasses *(19).* CPC is applied at a concentration not to exceed 0.3 g/lb (0.7 g/kg) of raw poultry carcass and in a solution that contains propylene glycol at a concentration 1.5 times that of CPC. The solution is applied as a fine spray at ambient temperature to raw poultry carcasses prior to immersion in a chiller. To reduce the possibility of the additive becoming a component of the food, and thus avoid additional exposure to humans who consume poultry, the petitioner proposed a system that ensures the capturing and recycling of the additive and disposal of residual CPC in a manner appropriate for FDA approval *(19).*

CPC is a quaternary ammonium compound with antimicrobial activity against a variety of gram-negative oral bacteria and is approved as a Category I compound in mouthwashes at concentrations up to 0.1% *(6, 71).* Arritt et al. *(21)* examined the efficacy of 0.1 and 0.5% CPC, TSP (10%), and ASC (0.1%) to inactivate, reverse, or inhibit the attachment of *C. jejuni* applied to chicken breast skin (28 cm^2). When bacteria were applied before treatment, reductions of 2.89 and 1.42 log CFU per skin were achieved with 0.5 and 0.1% CPC, respectively. When bacteria were applied after chemical treatment, a reduction of 4.67 log CFU per skin was achieved with 0.5% CPC. CPC (0.5%), TSP, and ASC were similarly effective for reducing *C. jejuni* populations on chicken skin. Both 10% TSP and 0.1% ASC were more effective as antimicrobials with longer contact time on chicken skin (3 or 10 min versus 0.5 min), especially when they were used prior to *C. jejuni* application. CPC at 0.5% was the most effective antimicrobial agent, with a 99.7% reduction of *C. jejuni.* However, 0.1% CPC was generally less effective than 10% TSP or 0.1% ASC for inactivating, reversing, and inhibiting attachment of *C. jejuni* to chicken skin *(21).*

Sodium hypochlorite (chlorine). Chlorine is the compound with the longest history of use in the United States to prevent cross-contamination of carcasses with bacteria during immersion chilling. Low cost and availability are the major reasons for the widespread use of chlorine in poultry processing *(75).* Free chlorine is the key substance that provides bactericidal activity. The amount of residual free chlorine in chilled water depends on the amount of chlorine added, the organic load in the chilled water, and the contact time *(75).* Chlorine demand is the maximum amount of chlorine that a solution can consume. Chilled water would require more than 400 ppm of chlorine to saturate the demand of organic compounds that react with chlorine *(75).* Chlorination of carcass wash water at 230 ppm produced noticeable concentrations of chlorine in the air in the vicinity of the washer, but did not reduce the aerobic plate count and coliform numbers compared with water that had 40 to 60 ppm chlorine *(66).* Ultimately, the effectiveness of chlorine as an antibacterial compound is highly questionable because the effect of chlorine is quickly counteracted by organic material present in chill water *(76),* and because no

free chlorine appears to be available to affect *Salmonella* already attached to the carcass *(44)*.

The use of chlorine in poultry processing has been a source of confusion for the industry after the establishment of the hazard analysis and critical control point (HACCP) systems *(4)*. The FSIS has now stated that up to 50 ppm available chlorine, measured at intake, can be added to water used to initially fill the prechiller, chiller, or reuse water system (red water), or to water added as makeup water *(2, 17)*. The FSIS has defined reuse water as ''water, ice, and solutions previously used to chill or wash raw product which may be reused provided that measures are taken to reduce physical, chemical, and microbiological contamination so as to prevent contamination or adulteration of product, and follow 9 CFR 416.2(g)'' *(17)*. Water reused in the prechiller or chiller may contain up to 5 ppm free available chlorine measured at influent to the prechiller or chiller *(17)*. The FSIS has specified that ''within these levels, the chlorine is to be used in an amount that does not exceed the minimum required to accomplish its intended effect'' and cited the study by Sanders and Blackshear *(66)* as an example. The FSIS expects each establishment to demonstrate that these levels are not exceeded by maintaining records as part of an HACCP plan, sanitation standard operating procedure, or prerequisite program *(17)*.

Yang et al. *(79)* found that increasing the age (in hours) of the water used for chilling decreased significantly the antibacterial effect of chlorine. New chilled water with 10 ppm of chlorine reduced *C. jejuni* by 3.3 log CFU/ml of chill water, whereas chilled water with 10 ppm of chlorine that had been used for 8 h reduced *C. jejuni* by less than 0.5 log CFU/ml of chill water. In these experiments, chlorination of chilled water did not effectively reduce the bacteria attached to chicken skins.

ClO_2. Chlorine dioxide (ClO_2) is an oxidizing biocide that destroys microorganisms by direct action on the cellular membrane and through oxidation of cellular constituents *(15, 67)*. ClO_2 disrupts the permeability of the outer membrane, penetrates bacterial cells, and disrupts protein synthesis *(23, 30)*. ClO_2 is less affected by pH and organic matter than is chlorine. It has an oxidizing power 2.5 times higher than that of chlorine, and it does not react with ammonia to form chloramines. However, ClO_2 reacts with reduced sulfur compounds, secondary and tertiary amines, and highly reduced and reactive organic compounds *(15)*. The FDA has approved the use of up to 3 ppm residual ClO_2 to control microbial populations in poultry process water contacting whole fresh poultry carcasses *(7)*. Because ClO_2 is unstable, a requisite for its effectiveness has been its generation on site. But the development in recent years of technologies that permit shipment to points of use makes ClO_2 a more popular disinfectant *(30)*.

Doyle and Waldroup *(38)* found that ClO_2 added to chill water in two commercial broiler processing plants reduced *Campylobacter* spp. concentrations by 90%. The efficacy of ClO_2 may be affected by large amounts of organic material in chilled water *(75)*, which may explain the inconsistent results for *Salmonella* and *Listeria* spp. *(38)* on carcasses immersed in chilled water with 1 to 3 ppm of ClO_2 *(15)*.

Ozone. Ozone has been affirmed by an independent panel of experts sponsored by the Electric Power Research Institute *(41)* as GRAS for use as a sanitizer for foods. The use must comply with concentrations and application methods consistent with good manufacturing practices *(41)*. The FDA has reaffirmed the GRAS status of ozone *(8)* and amended the food additive regulations to provide for the safe use of ozone in gaseous and aqueous phases as an antimicrobial agent on food, including meat and poultry *(13)*.

The main inactivation mechanism of ozone against *E. coli* is the direct reaction with cellular compounds *(73)*. Indirect reaction with free radicals also has been suggested as a mechanism for inactivation of *E. coli (73)*. Ozone has an oxidizing effect 1.5 times stronger than that of chlorine *(78)*. It autodecomposes and it leaves no toxic residue, all of which make ozone a good alternative for disinfection of process water *(41, 78)*. Ozone appears to be more effective than chlorine for destroying parasites (*Giardia* and *Cryptosporidium*) in process water *(41)*. The death rate among gram-negative bacteria is not affected by the presence of organic material, but the addition of 20 ppm serum albumin reduces the killing power of ozone *(61)*. The applications and potential uses of ozone in the food industry have been reviewed by Kim et al. *(48)*. Several ozone applications are now available to treat the water used in the poultry and the produce industry *(78)*. Yang and Chen *(80)* suggested that ozone preferentially destroys gram-negative rods. Sheldon and Brown *(68)* found that ozone may be effective in reducing bacterial numbers in chiller water, and treatment of spent chiller water is the major opportunity for use of ozone by the poultry industry. The treatment and recycling of spent chiller water can significantly reduce the total water usage by processing plants. There is no available information on the killing power of ozone against *Campylobacter* spp.

Peroxyacids. On 19 September 2001, the FDA amended the food additive regulations to provide for the safe use of a mixture of peroxyacetic acid, octanoic acid, acetic acid, hydrogen peroxide, peroxyoctanoic acid, and 1-hydroxyethylidene-1,1-diphosphonic acid as an antimicrobial agent on poultry carcasses, poultry parts, and organs *(14)*. The additive is now codified under 21 CFR section 173.370 and can be used as an antimicrobial agent on poultry carcasses, poultry parts, and organs in accordance with current industry standards of good manufacturing practice (unless precluded by USDA standards of identity in 9 CFR part 381, subpart P), where the maximum concentration of peroxyacids is 220 ppm as peroxyacetic acid, the maximum concentration of hydrogen peroxide is 110 ppm, and the maximum concentration of 1-hydroxyethylidene-1,1-diphosphonic acid is 13 ppm *(14)*.

The strong oxidizing function of peroxyacetic acid, hydrogen peroxide, and peroxyoctanoic acid disrupts the permeability of cell membranes and alters protein synthesis through reactions with sulfhydryl, sulfide, amino acids con-

taining disulfide, and nucleotides. Indirect antimicrobial actions occur through the acidification of the carcass surface and the penetration of undissociated acids into the bacterial cell *(16).* A manufacturing company has submitted an in-plant trial validation protocol to the FSIS to allow the product to be used for on-line reprocessing.

An efficacy study of peroxyacids for poultry decontamination has not been published in peer-reviewed journals. Two studies (lab-scale and in-plant) commissioned by a private laboratory provide the only available information, but interpretation of the results for the spray applications must take into consideration that a number of the microorganisms are removed by the mechanical effect of spraying. Therefore, the efficacy associated with only the chemical agent should be estimated by subtracting the bacteria mechanically removed by using a spray control treatment with water only *(16).* Evaluation of any kind of application delivered in spray or dip tank should include a control treatment with water only (same temperature and volume) to determine the efficacy strictly associated with the chemical agent.

TSP. A mixed solution of TSP has a pH of 10 to 12 *(16, 65).* The high alkalinity of TSP appears to remove fat films and bacteria and to disrupt fatty molecules in the cell membrane, causing the bacterial cells to leak intracellular fluid *(40, 65).* Some research suggests that the main mechanism of action of TSP on *Salmonella* is the detachment of contaminants from the skin surface *(49),* but the details of the antimicrobial mechanism have not been completely elucidated *(42).* One major limitation for widespread use of TSP is the increase in the phosphate concentration of the waste water.

The FDA has approved TSP as a multiple-purpose GRAS food substance when used in accordance with good manufacturing practices *(11).* The FSIS has approved TSP as an antimicrobial agent to be used on raw chilled (amended to include prechilled) poultry carcasses at 8 to 12% in conjunction with a water spray containing 20 ppm chlorine. The TSP solution must be maintained between 45 and 55°F after chilling and applied by spraying or dipping chilled or prechilled carcasses for up to 15 s *(5).* TSP has received USDA approval for use in on-line reprocessing *(12).*

Slavik et al. *(70)* studied the effects of postchill dip applications of TSP on the control of *Campylobacter* spp. over time. Carcasses were dipped into a 10% solution of TSP at 50°C for 15 s and then stored at 4°C for 0, 1, or 6 days before they were analyzed for *Campylobacter.* A nontreated control group of carcasses was also stored under similar conditions for comparison. At day 0, there was no reduction in *Campylobacter* numbers compared with nontreated controls. After 1 day of storage, there was a 4 to 36% reduction of *Campylobacter* for treated carcasses, based on results of a standard culture method. Quantification of the reduction by a most probable number method resulted in a *Campylobacter* reduction of 1.5 and 1.2 log CFU in TSP-treated carcasses stored for 1 and 6 days, respectively. Salvat et al. *(64)* also reported a reduction in the incidence and numbers of *Campylobacter* on chicken neck skin samples after treatment with a 10% solution of TSP. Somers et al. *(72)* studied the effectiveness of TSP against suspended and attached (biofilm) cells of *C. jejuni, E. coli* O157:H7, *L. monocytogenes,* and *Salmonella* Typhimurium. At room temperature (22°C) and at 10°C, *E. coli* O157:H7 was the most sensitive organism to TSP treatments and *C. jejuni* was slightly less sensitive. These results suggested that TSP is effective for reducing suspended populations of *C. jejuni, E. coli* O157:H7, and *Salmonella* Typhimurium, but that biofilm cells of *Salmonella* Typhimurium and *L. monocytogenes* are more resistant to TSP at 10°C *(72).* Other results suggest that *C. jejuni* cells exposed to sublethal concentrations of TSP (0.5 to 5 mM for 10 min) may have increased sensitivity to lysozyme and nisin *(33).* The information presented to the FSIS by a TSP manufacturer included a reduction in numbers of *Campylobacter* of 4 log CFU and a reduction in prevalence of 30 to 100% *(16).* However, a comprehensive review by Capita et al. *(34)* of studies published in peer-reviewed journals revealed that laboratory and in-plant applications of TSP reduced bacteria in poultry by 1 to 2 log CFU.

SUMMARY

In general, the application of the described technologies results in a reduction in *Campylobacter* spp. of 1 to 2 log CFU/ml of carcass rinse (Table 2). However, postchill *Campylobacter* concentrations of 0.5 and 1 log CFU/ml of carcass rinse, which represents approximately 4,000 CFU per carcass, are still common *(59).* In addition to the technologies described, there are other interventions, such as ionizing radiation *(60),* the combination of lactic acid and sodium sorbate *(42),* or acetic acid *(56),* that can be used to reduce *Campylobacter* spp. However, the use of these other interventions has been limited by their cost or the adverse sensory changes that result from their application. For a single decontamination technology to be more than 99.9% effective in reducing *Campylobacter* spp., it would have to consistently reduce 3.7 log CFU/ml of carcass rinse after evisceration and throughout postchill with minimal impact on the organoleptic characteristics of the final product *(47).*

The lack of a standard methodology for *Campylobacter* isolation and enumeration has always been a problem when attempting to understand the prevalence and counts of *Campylobacter* spp. in poultry carcasses. To assess the full potential of an antimicrobial application, we must have a solid microbiology technique for enumeration of *Campylobacter* spp. that could be used under different conditions. Our current isolation methods may perform differently when counting *Campylobacter* numbers postchill. In a recent study, the most effective methods available for direct postchill enumeration of *Campylobacter* were assessed *(59),* but we do not know if these methods are all similarly effective for *Campylobacter* in prechilled (pre- or postdefeathered and pre- or posteviscerated) carcasses. Effective methodologies for *Campylobacter* enumeration are an important tool for studying interventions or process steps aimed at reducing *Campylobacter* numbers on poultry carcasses.

TABLE 2. *Effectiveness of commercial antimicrobial agents for reducing* Campylobacter *spp. on commercial broiler chicken carcasses*

Antimicrobial	Reduction of *Campylobacter* spp.
Acidified sodium chlorite	1.75 log CFU/ml of carcass rinse *(45)*; 99.2% by the combined effect of bird washers and prechill spray *(47)*; 0.2 log CFU/ml of carcass rinse after postchill dip application *(58)*
Cetylpyridinium chloride	In vitro studies: 2.89 log CFU per skin with 0.5% CPC; 1.42 log CFU per skin with 0.1% CPC; 99.7% with 0.5% CPC *(21)*
Chlorine	6 log CFU/ml in new chilled water with 30 ppm of chlorine (10 min); 5 log CFU/ml in 8-h chilled water with 50 ppm of chlorine (50 min); chlorination of chilled water did not reduce attached bacteria *(79)*
Chlorine dioxide	90% when added to chill water in commercial broiler processing plants *(38)*
Ozone	No available information in the scientific literature
Peroxyacids	No available information in the scientific literature *(16)*
Trisodium phosphate	4 log CFU and 30–100% reduction in prevalence, presented to FSIS by a manufacturer *(16)*; 1–2 log CFU for laboratory and in-plant applications and for several bacteria *(34)*. No reduction at day 0, but 4–36% reduction (1.5 log CFU reduction per carcass) at day 1 by 10% postchill dip applications at 50°C for 15 s *(70)*

Another area that requires consideration is the potential development of resistant *Campylobacter* populations resulting from the use of sublethal concentrations of biocides. We do not have a complete understanding of the appearance or lack of appearance of these stressed populations, nor do we know if these stress-adapted *Campylobacter* cells can survive under harsh environmental conditions and thus pose a greater threat to humans. Any potential adaptation responses that *Campylobacter* may develop in response to different antimicrobial agents should be further studied.

We also must improve our understanding of how effective antimicrobials are for destroying attached bacteria and how food contact surfaces may play a role in spreading *Campylobacter* contamination during processing *(43)*. The extent of contamination can be measured only by collecting more in-plant data to determine changes in *Campylobacter* carriage by the product throughout the different processing steps.

The poultry industry should be receptive to new interventions that could be applied at different stages during processing. Applications such as mists, sprays, or baths, which can be applied closer to the final stages in processing, may result in a final product with less *Campylobacter* contamination. Even the scheduled delivery of *Campylobacter*-free chicken flocks to be processed first should be evaluated as a tool to keep clean products segregated as much as possible during processing. Many poultry processors have increased the use of chlorine and water and have incorporated intervention strategies to reduce *E. coli* and *Salmonella* on carcasses to comply with the pathogen reduction and HACCP regulations *(4)*. However, these strategies may have little impact on the reduction of *Campylobacter* populations. The high *Campylobacter* numbers found on carcasses postchill is still a concern because of the low infective dose (500 cells) that has been reported to produce human disease from contaminated milk *(50, 63)*. We do not know whether a similar infective dose may apply to poultry products. Development of new interventions postchill, an understanding of possible resistance to antimicrobial agents, improvements in detection methodologies, and additional surveys of the contamination of processed poultry will translate into better control of *Campylobacter* in commercial poultry carcasses.

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